

Claims

1. An isolated DNA molecule comprising a nucleotide sequence  
5 that encodes a biologically active protamine polypeptide  
or functional fragment thereof including an amino acid  
sequence selected from the group consisting of:
  - 10 (a) amino acid sequences extending from position 9 to  
position 18, from position 9 to position 20, from  
position 9 to position 21, from position 9 to  
position 22, from position 9 to position 23, from  
position 10 to position 20, from position 10 to  
position 21, from position 10 to position 22, from  
15 position 10 to position 23, and from position 10 to  
24 of SEQ ID NO. 2; and
  - (b) amino acid sequences as set out in SEQ ID NOS. 14,  
16, 18, 20, 22, 24, and 26.
- 20 2. An isolated DNA molecule comprising a nucleotide sequence  
that encodes a biologically active protamine polypeptide  
or functional fragment thereof, wherein the nucleotide  
sequence is selected from the group consisting of:
  - 25 (a) nucleic acid sequences encoding amino acid sequences  
extending from position 9 to position 18, from  
position 9 to position 20, from position 9 to  
position 21, from position 9 to position 22, from  
position 9 to position 23, from position 10 to  
30 position 20, from position 10 to position 21, from  
position 10 to position 22, from position 10 to

position 23, and from position 10 to 24 of SEQ ID NO.  
2; and

(b) nucleic acid sequences as set out in SEQ ID NOS. 13,  
15, 17, 19, 21, 23, and 25; and

5 (c) nucleic acid sequences as set out in SEQ ID NOS. 28,  
29, 30, 31, and 32.

10 3. An isolated DNA molecule providing an expression cassette  
capable of directing the expression of a biologically  
active protamine polypeptide or functional fragment  
thereof in a suitable host, wherein said expression  
cassette comprises from 5' to 3':

15 (a) a promoter capable of expressing a downstream coding  
sequence in a suitable host;

(b) a DNA sequence coding for the expression of a  
biologically active protamine polypeptide or  
functional fragment thereof; and

(c) a 3' termination sequence.

20

4. Isolated DNA molecule according to claim 3, wherein the  
DNA sequence (b) is selected from the group consisting  
of:

25 (a) a nucleotide sequence that encodes a biologically  
active protamine polypeptide or functional fragment  
thereof including an amino acid sequence selected  
from the group consisting of:

30 (i) amino acid sequences extending from position 9  
to position 18, from position 9 to position 20,  
from position 9 to position 21, from position 9

to position 22, from position 9 to position 23,  
from position 10 to position 20, from position  
10 to position 21, from position 10 to position  
22, from position 10 to position 23, and from  
position 10 to 24 of SEQ ID NO. 2;

(ii) amino acid sequences as set out in SEQ ID NOS.  
2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, and  
26;

(iii) amino acid sequences derived from SEQ ID NO.  
33;

(b) nucleic acid sequences as set out in SEQ ID NOS. 1,  
3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, and 25; and

(c) nucleic acid sequences as set out in SEQ ID NOS. 27,  
28, 29, 30, 31, and 32.

5. Isolated DNA molecule according to any of the preceding  
claims, wherein the coding nucleotide sequence is a cDNA,  
genomic or manufactured DNA sequence.

6. Isolated DNA molecule according to any of claims 3 to 5,  
wherein the coding nucleotide sequence is fused with a  
suitable signal peptide encoding sequence.

7. Isolated DNA molecule according to any of claims 3 to 6,  
wherein the promoter and/or the coding nucleotide  
sequence(s) are selected to ensure expression in an  
eucaryotic host.

8. Isolated DNA molecule according to any of claims 3 to 6, wherein the promoter and/or the nucleic acid sequence are selected to ensure expression in a procaryotic host.
- 5 9. Isolated DNA molecule according to claim 7 or 8, wherein the promoter is an inducible promoter.
10. A plasmid or vector system comprising one or more DNA molecules according to any of claims 1 to 9.
- 10 11. A procaryotic or eucaryotic host cell, seed, tissue or whole organism transformed or transfected with the DNA molecule according to any of claims 3 to 9 or with the plasmid or vector system according to claim 10 in a manner enabling said host cell, seed, tissue or whole organism to express a protamine polypeptide or functional fragment thereof.
- 15 12. The procaryotic or eucaryotic host cell, seed, tissue or whole organism according to claim 11 selected from the group consisting of bacteria, fungi including yeast, insect, animal and plant cells, seeds, tissues or whole organisms.
- 20 13. The procaryotic host cell or whole organism according to claim 12 being a bacterium selected from the group consisting of proteobacteria including members of the alpha, beta, gamma, delta and epsilon subdivision, gram-positive bacteria including Actinomycetes, Firmicutes, Clostridium and relatives, flavobacteria, cyanobacteria, green sulfur bacteria, green non-sulfur bacteria, and archaea.
- 25 30

14. The procaryotic host cell or whole organism according to claim 13 belonging to the group of proteobacteria selected from the group consisting of *Agrobacterium*,  
5 *Rhodospirillum*, *Rhodopseudomonas*, *Rhodobacter*, *Rhodomicrobium*, *Rhodopila*, *Rhizobium*, *Nitrobacter*, *Aquaspirillum*, *Hyphomicrobium*, *Acetobacter*, *Beijerinckia*, *Paracoccus*, *Pseudomonas*, ammonia-oxidizing bacteria such as *Nitrosomonas*, *Enterobacteriaceae*, *Myxobacteria* such as  
10 *Myxococcus*, with *Rhodopseudomonas*, *Pseudomonas* and *Escherichia* being preferred, and with *Rhodopseudomonas palustris*, *Pseudomonas fluorescens*, and *Escherichia coli*, respectively, being most preferred.
- 15 15. The procaryotic host cell or whole organism according to claim 13 belonging to the group of gram-positive bacteria selected from the group consisting of *Actinomycetes* and *Firmicutes* including *Clostridium* and relatives such as *Bacillus* and *Lactococcus*, with *Bacillus subtilis* and  
20 *Lactococcus lactis* being preferred.
16. The procaryotic host cell or whole organism according to claim 13 belonging to the group of flavobacteria selected from the group consisting of *Bacteroides*, *Cytophaga* and  
25 *Flavobacterium*, with *Flavobacterium* such as *Flavobacterium* ATCC21588 being preferred.
17. The procaryotic host cell or whole organism according to claim 13 belonging to the group of cyanobacteria selected from the group consisting of *Chlorococcales* including  
30 *Synechocystis* and *Synechococcus*, with *Synechocystis* sp. and *Synechococcus* sp. PS717 being preferred.

18. The procaryotic host cell or whole organism according to claim 13 belonging to the groups of green sulfur bacteria or green non-sulfur bacteria selected from Chlorobium or Chloroflexaceae such as Chloroflexus, respectively, with Chlorobium limicola f. thiosulfatophilum and Chloroflexus aurantiacus, respectively, being preferred.
19. The procaryotic host cell or whole organism according to claim 13 belonging to the group of archaea selected from Halobacteriaceae such as Halobacterium, with Halobacterium salinarum being preferred.
20. The eucaryotic host cell or whole organism according to claim 12 being fungi including yeast selected from the group consisting of Ascomycota including Saccharomycetes such as Pichia and Saccharomyces, and anamorphic Ascomycota including Aspergillus, with Saccharomyces cerevisiae and Aspergillus niger being preferred.
21. The eucaryotic host cell according to claim 12 being an insect cell selected from the group consisting of SF9, SF21, Trychplusiani and MB21.
22. The eucaryotic host cell according to claim 12 being an animal cell selected from the group consisting of Baby Hamster Kidney (BHK) cells, Chinese Hamster Ovarian (CHO) cells, Human Embryonic Kidney (HEK) cells and COS cells, with NIH 3T3 and 293 being most preferred.
23. The eucaryotic host cell, seed, tissue or whole organism according to claim 12 being a plant cell, seed, tissue or

whole organism selected from the group consisting of eukaryotic alga, embryophytes comprising *Bryophyta*, *Pteridophyta* and *Spermatophyta* such as *Gymnospermae* and *Angiospermae*, the latter including *Magnoliopsida*,  
5 *Rosopsida*, and *Liliopsida* ("monocots").

24. A method of transforming or transfecting a prokaryotic or eucaryotic host cell, seed, tissue or whole organism according to any of claims 11 to 23 in order to yield  
10 transformants or transfectants capable of expressing a protamine polypeptide or functional fragment thereof, comprising the transformation or transfection of said host cell, seed, tissue or whole organism with a DNA molecule according to any of claims 3 to 9, or with a  
15 plasmid or vector system according to claim 10.

25. A transformed or transfected host cell, seed, tissue or whole organism represented by or regenerated from transformants or transfectants yielded according to claim  
20 24.

26. Method for the production of a biologically active protamine polypeptide or functional fragment thereof, comprising the steps of:

25 (a) culturing a transformed or transfected host cell, seed, tissue or whole organism according to claim 25 under suitable conditions allowing production of said polypeptide or functional fragment within said host;  
30 and, optionally,

(b) isolating said polypeptide or functional fragment from said host or from its culture medium.

27. Method according to claim 26, wherein said transformed or transfected host cell is selected from prokaryotes according to any of claims 11 to 19, with  
5 Rhodopseudomonas palustris, Pseudomonas fluorescens, and Escherichia coli being preferred, and wherein said polypeptide or functional fragment is isolated after induction of a log phase culture with a suitable inducing agent.
- 10 28. Method according to claim 27, wherein said polypeptide or functional fragment is isolated until said host cell re-enters log phase.
- 15 29. Bacteriocidal, bacteriostatic, fungicidal and/or fungistatic composition comprising or consisting essentially of citrate and bicarbonate.
- 20 30. Composition according to claim 29, further comprising phosphate.
- 25 31. Composition according to claim 29 or 30, further comprising a basic protein or peptide selected from the group consisting of protamines, protamine sulphates, defensins, magainins, mellitin, cecropins and protegrins.
32. Composition according to claim 31, wherein the basic protein or peptide is protamine or protamine sulphate.
- 30 33. Composition according to claim 32, wherein a suitable biologically active protamine or functional fragment thereof can be selected from the group consisting of



proteins, polypeptides or peptides representing or comprising amino acid sequences extending from position 9 to position 18, from position 9 to position 20, from position 9 to position 21, from position 9 to position 22, from position 9 to position 23, from position 10 to position 20, from position 10 to position 21, from position 10 to position 22, from position 10 to position 23, and from position 10 to 24 of SEQ ID NO. 2; amino acid sequences as set out in SEQ ID NOS. 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, and 26; amino acid sequences derived from SEQ ID NO. 33; amino acid sequences being encoded by nucleic acid sequences as set out in SEQ ID NOS. 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, and 25; and amino acid sequences being encoded by nucleic acid sequences as set out in SEQ ID NOS. 27, 28, 29, 30, 31, and 32.

34. Composition according to any of claims 29 to 33, further comprising lysozyme.

35. Composition according to any of claims 29, and 31 to 34, wherein citrate and bicarbonate are present in a molar ratio of 4:1, and wherein the preferred amounts of citrate and bicarbonate are at least 0.04 M and 0.01 M, respectively.

36. Composition according to any of claims 30 to 34, wherein citrate, bicarbonate and phosphate are present in a molar ratio of 2:1:1, and wherein the preferred amounts of citrate, bicarbonate and phosphate are at least 0.2 M, 0.1 M and 0.1 M, respectively.

37. Composition according to any of claims 31 to 36, wherein the basic protein or peptide is present in an amount of at least 0.1 µg.

5 38. Composition according to any of claims 32 to 37, wherein lysozyme is present in an amount of at least 0.1 µg.

39. Composition according to any of claims 29, 31 to 35, 37 and 38 in liquid form, obtained by admixing an at least  
10 0.01 mol/l citrate solution adjusted to pH 6.0 to 7.0 with an at least 0.01 mol/l bicarbonate solution in a ratio of 4:1 (vol./vol.).

40. Composition according to any of claims 30 to 34, and 36  
15 to 38 in liquid form, obtained by admixing an at least 0.01 mol/l citrate solution adjusted to pH 6.0 to 7.0 with an at least 0.01 mol/l bicarbonate solution and an at least 0.01 mol/l phosphate solution adjusted to pH 6.0 to 7.0 in a ratio of 2:1:1 (vol./vol.).

20 41. Composition according to claim 39 or 40 in liquid form, comprising protamine in a concentration of at least 0.1 µg/ml.

- 25 ~~42. Composition according to any of claims 39 to 41, comprising Lysozyme in a concentration of at least 0.1 µg/ml.~~

30 43. Protamine polypeptide or peptide having amino acid sequences extending from position 9 to position 18, from position 9 to position 20, from position 9 to position 21, from position 9 to position 22, from position 9 to

position 23, from position 10 to position 20, from position 10 to position 21, from position 10 to position 22, from position 10 to position 23, or from position 10 to 24 of SEQ ID NO. 2.